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Effects of Broiler Rearing Environment on Transmission of F-Strain *Mycoplasma gallisepticum* from Commercial Layer Hens to Broiler Chickens: Role of Acid-Base Balance

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Abstract: Two trials were conducted concurrently to determine and compare, blood pH, blood gases, hematocrit and hemoglobin in F-strain *Mycoplasma gallisepticum* (FMG) inoculated layers and FMG contact-infected broilers. At the termination of the study, FMG-inoculated layers had the highest partial pressure of O_2 and the lowest partial pressure of CO_2 as compared with the other treatment groups. Blood pH values were unaffected by FMG inoculation. Hematocrit and blood concentrations of hemoglobin were slightly higher and HCO_3^- levels were lowest in FMG contact-infected broilers in comparison to the other treatments groups. *Mycoplasma gallisepticum* inoculated layers also resulted in a significant increase in blood concentrations of K^+, a decrease in Na^+, but no significant effects on blood concentrations of Ca^{2+} and Cl^-. There were no differences in plasma glucose, cholesterone, triglyceride and anion gap, but osmolality was significantly reduced in FMG contact-infected broilers. Results indicate that inoculation of layers with FMG vaccine results in changes in plasma acid-base status along with changes in other blood metabolic variables. However, the FMG inoculation did not prevent homeostatic regulation of acid-base balance, as indicated by constant blood pH. The significant increase in pO_2 in FMG inoculated layers is generally associated with an oxygen-dependent improvement in tissue oxygenation. Elevated arterial partial pressure of oxygen is beneficial to maximize oxygen transport capacity along with high concentrations of hemoglobin and hematocrit to carry oxygen throughout the body. It was concluded that in addition to protecting birds from MG infection, an FMG vaccine may improve the layer chicken's ability to withstand the harmful effects of stressors on their performance and well-being.

Key words: *Mycoplasma gallisepticum*, acid-base balance, broiler chickens

INTRODUCTION

Mycoplasma Gallisepticum (MG) infection in commercial layers is common in many parts of the world. *Mycoplasma gallisepticum* infection can be spread short distances by the air-borne route (Butcher, 2002). The disease is predominantly spread from farm to farm by movement of contaminated equipment, vehicles and people. It has been reported that MG is the most important mycoplasma species among the agents of infection and disease in poultry (Simmons *et al*., 1997), causing great economic and production losses in the poultry industry. It is not only a primary disease agent in layers, but it also persists in broiler breeders and broilers worldwide. Egg transmission to broiler progeny occurs at a low level from infected breeders (Butcher, 2002), while horizontal infection readily occurs in broiler houses. *Mycoplasma gallisepticum* may not cause obvious signs of disease in chickens unless the birds are stressed with respiratory viruses such as Infectious Bronchitis (Cavagh, 2007).

It has been shown that the mycoplasma pathogen is able to spread throughout the body following aerosol infection, as demonstrated by re-isolation of MG from the heart, kidneys, brain, spleen and liver of experimentally infected chickens (Much *et al*., 2002). How this agent manages to convert a local infection into a systemic infection remains unknown. Erythrocyte-invasive organisms have been detected not only after *in vitro* infection but also *in vivo* in blood samples from experimentally infected chickens (Gunther *et al*., 2008), suggesting that there is an infection strategy that was previously unknown for pathogenic mycoplasmas. Despite the wide reports on blood characteristics and chemistry of MG infected layer hens (Siegel *et al*., 1972; Burnham *et al*., 2003; Peebles *et al*., 2006; Gunther *et al*., 2008), there is no available information on physiological blood gas parameters and acid-base balance. The precise action of the immunological effects associated with acid-base balance is poorly understood. In addition, in many cases it is not known whether the normal blood physiological variable values that maximize productivity in healthy and unchallenged birds are optimal for immunocompetence and disease resistance. Believing that a better knowledge of blood gas parameters and acid-base balance throughout the experiment could be important in assessing experimental findings and for monitoring physiological status, blood gas parameters and acid-base balance

were investigated in MG inoculated layer hens and contact-infected broilers to assess the possible influence of acid-base balance on blood physiological variables.

MATERIALS AND METHODS

Housing and management: Two concurrent trials were conducted in this study and a completely randomized experimental design was utilized. In each trial, 120 1-d-old (Ross × Ross 708) broiler chicks were purchased from a commercial hatchery (Aviagen, Inc., Huntsville, AL). Chicks were vaccinated for Mareks, Newcastle and Infectious Bronchitis at the hatchery. In addition, eight commercial layer pullets were obtained from a commercial source and demonstrated MG-free via serological test (SPA) and choanal cleft culture (Butcher, 2002). Broiler chicks were randomly placed in a total of six floor pens (20 chicks/pen) per trial with the exception of a single pen in each trial in which 16 broilers were placed along with four layer pullets. The research facility contained two rooms separated by a solid wall with sealed doors (designated North and South), both containing 32 floor pens each. The pens containing both the layers and broilers were located on the opposite end of the room from the exhaust fans in each room. [All pens measured 1.5 × 2.7 m (5 × 9 ft)]. The commercially obtained layer chickens were inoculated via eyedrop in the right eye with 0.04 mL (1 dose) of commercial F-strain *Mycoplasma Gallisepticum* (FMG) vaccine ([Fvax-MG®] Fort Dodge Animal Health, Princeton, NJ). Floor pens were identical in floor space, feeder space and waterer space per bird and lighting (intensity and duration). Each pen was equipped with a tube feeder, nipple watering system having seven nipples and fresh pine shavings. Birds had free access to feed and water for ad libitum consumption.

All birds were provided a broiler 3-phase feeding program (starter, grower, finisher). Diets were formulated to meet or exceed NRC (1994) nutrient recommendations and all trials were conducted under an approved USDA animal care and use protocol. Ambient temperature was maintained at 33°C at the start of experimentation and reduced as the birds progressed in age, with a final temperature of 21°C at 35 d and thereafter. The incidence of mortality was recorded together with pen origin and necropsies were conducted on all birds that died during the trials.

At nine weeks and two days of age in Trial 1 and at nine weeks and three days of age in Trial 2, ten randomly selected pullets were bled from the left vena cutanea ulnaris to obtain blood for the serum to be tested for antibodies to both MG and MS, using the Serum Plate Agglutination (SPA) (Kleven, 1981). At those same times, swabs were also collected from the choanal cleft (Butcher, 2002) and placed into tubes containing Frey's-based (Papageorgiou medium) broth (Frey *et al*., 1968) supplemented with an additional 100 mg/L of thallium acetate and 1.5 millions IU/L penicillin G. Tubes were incubated at 37°C for 30 d or until the phenol red indicator reaction occurred in the media, indicating growth. Media samples from tubes that showed growth were inoculated onto Frey's based agar and incubated at 37°C. Colonies with morphology suggestive of Mycoplasma species were examined by an agar plate Fluorescent Antibody (FA) method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-MG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Habler *et al*., 1998).

Treatments: 1 = Broilers at 13.7 meters (45 ft) downwind from treatment 6; 2 = Broilers at 6.1 meters (20 ft) downwind from treatment 6; 3 = Broilers at 1.5 meters (5 ft) downwind from treatment 6; 4 = Broilers at 1.5 meters (5 ft) upwind from treatment 6; 5 = Broilers directly opposite treatment 6 and separated by a solid partition (41.9 cm high by 16.5 cm wide); 6 = Broilers penned with 4 FMG inoculated layers in treatment 7, 7 = 4 MG inoculated layers in treatment 6.

Blood collection and chemical analyses: On days 14 (Trial 1) and 42 (Trial 2), blood samples were collected as described previously (Olanrewaju *et al*., 2006, 2007, 2008) from a cutanea vlnaris vein of four randomly selected broilers from each pen including four broiler contact penmates in addition to the four inoculated layer pullets. Blood samples were collected directly into heparinized (50 IU/mL^{-1}) monovette syringes. All bleedings were completed within 45 s after birds were caught. Blood samples were drawn directly from the syringes into a blood gas/electrolyte analyzer (ABL-80 Flex, Radiometer America, Westlake, OH) for immediate analysis of Glucose (GLU), osmolality (mOsm), partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), pH, Hematocrit (Hct), Hemoglobin (Hb) and electrolytes (Na^+, K^+, Ca^{2+} and Cl). The pH, pCO_2 and pO_2 values were corrected to reflect a body temperature of 41.5°C (Burnett and Noonan, 1974). The needle mounted on each monovette syringe was then removed, a cap was placed over the needle port and the syringes containing the blood samples were submerged into ice.

After all birds were bled, the iced samples were transferred to the laboratory, centrifuged at 4000 × g for 20 min and the packed blood cells were expelled from the syringes. The plunger on each monovette was broken off and the syringe served as a storage vial for the remaining plasma. This procedure insured that the plasma samples were never exposed to ambient air. Plasma samples were stored at -20°C for later chemical analyses. Plasma samples were removed from the freezer, thawed and each sample was analyzed for Cholesterol (CHOL) and Triglyceride (TRIG) using an

auto analyzer (Vitro DT 6011, Ortho-Clinical Diagnostic, Rochester, NY). This analyzer employs enzymatic procedures that have been described by Elliott (1984). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

Statistical analyses: A completely randomized experimental design was utilized. The data of both trials were pooled and then analyzed together. Trial was taken to be a random effect. Individual sample data within each replicate unit were averaged before analysis. Results from Trials 1 and 2 were not reported independently but were reported over both trials. Least squares means were compared in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2004). Statements of significance were based on $p \leq 0.05$, unless otherwise stated.

RESULTS

Initial culture results from the eight layer chickens showed no evidence of mycoplasmal infection pre-vaccination. At the termination of the study, 12 of the 16 broilers housed with FMG infected layers as well as each of the four layers in both trials demonstrated MG seroconversion via SPA test. Culture results yielded MG from 10 of the 16 broilers housed with FMG infected layers as well as each of the four layers in both trials. However, other broilers in the study test did not yield MG seroconversion subsequent to culture of either the trachea or choanal cleft. No other broilers in the study either seroconverted or yielded MG subsequent to culture of either the trachea or choanal cleft. Blood pH, blood gases, hematocrit and hemoglobin in broilers and MG inoculated layers according to treatment are presented in Table 1. Blood pH value of MG inoculated layers was not statistically different compared with the other treatments. Blood pCO_2 level was lowest in MG inoculated layers and different only from treatment 4 which had the highest pCO_2 level, but not statistically differ from treatments 1, 2, 3, 5 and 6. *Mycoplasma gallisepticum* inoculated layers had significantly higher blood pO_2 than treatments 2, 3 and 4, but did not differ from treatments 1, 5 and 6. Blood HCO_3^- level was significantly lower in MG inoculated layers compared with treatments 1, 4 and 6, whereas the level did not differ from treatments 2, 3 and 5. Hematocrit value along with blood concentrations of hemoglobin were not statistically affected overall, but both were slightly higher in MG inoculated pullets.

Compared with the other treatments, the blood concentration of K^+ in the MG inoculated layers was significantly higher (Table 2). Level of Na^+ in MG inoculated layers was significantly lower than that of broilers in treatments 3, 4 and 5 groups, but not significantly different from treatments 1, 2 and 6. In addition, no significant effects on blood concentrations of Ca^{2+} and Cl^- were observed.

As shown in Table 3, osmolality level in the MG inoculated layers was significantly lower compared with treatments 3 and 4, but not statistically different from levels in treatments 1, 2, 5 and 6. There were no treatment differences in blood concentrations of GLU, CHOL, TRIG, or ANGAP.

DISCUSSION

The data reported here give the first comprehensive picture of the acid-base balance and oxygenation conditions in F-strain *Mycoplasma Gallisepticum* (FMG) inoculated layers and contact-infected broilers as compared with non-FMG infected broilers during rearing ventilation. The complete blood gas parameters and acid-base balance picture reported here closely resembles that reported for normal and healthy broiler chickens (Olanrewaju *et al*., 2006, 2007, 2008). This careful determining of pH, pCO_2, pO_2 and HCO_3^-, provides the opportunity to evaluate any change in the acid-base balance and to correct physiological alterations (i.e. acidosis or alkalosis).

The concentration of blood H^+ and other physiological variables are well regulated. Although pH is affected by numerous activities of the body, it is highly buffered to help keep free H^+ within physiological limits. In the current study, traditional acid-base variables (pH, pCO_2, HCO_3^-) using whole blood samples, indicated only a minimal plasma pH disturbance. Regulatory mechanisms, including increased renal acid excretion, effectively maintained plasma pH within normal ranges (Olanrewaju *et al*., 2006, 2007, 2008) throughout the experiment. Decreased blood pCO_2 along with concurrent increased blood pO_2 in MG inoculated layers indicated that MG vaccine may enhance overall immune response due to its direct and indirect effects on immune function. Significant increases in the levels of arterial pO_2 and decreases in pCO_2 have been reported in pneumocystis-infected mice (Qureshi *et al*., 2003). An increase in mixed venous pO_2 indicates improvement in global oxygenation status (Hogan *et al*., 1992). High pCO_2 has been reported to suppress immune response of various human peritoneal cells (Kopernik *et al*., 1998). High pO_2 is also substantiated by the fact that increases in mixed venous pO_2 are generally associated with an oxygen-dependent improvement in tissue oxygenation, as demonstrated in the heart, muscle, brain, liver and gut (Mosca *et al*., 1996; Much *et al*., 2002). Oxygen delivery depends on blood flow and arterial oxygen content, which in turn depends on arterial pO_2, the affinity of hemoglobin concentration to maximize the oxygen transport capacity along with high concentration hematocrit to carry oxygen throughout the body systems

Table 1: Influence of MG inoculation on blood pH, blood gases, hematocrit and hemoglobin in inoculated hens and broiler chickens

Treatment[1]	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3^- (mmHg)	Hct (%)	Hb (g/dL)
1	7.33[ab]	57.5[ab]	46.8[ab]	27.4[a]	23.4	7.48
2	7.33[ab]	55.2[ab]	42.4[b]	26.6[ab]	23.3	7.47
3	7.30[b]	60.5[ab]	45.7[b]	26.7[ab]	24.4	7.83
4	7.31[ab]	61.6[a]	43.4[b]	27.9[a]	24.6	7.91
5	7.35[ab]	53.5[ab]	51.7[ab]	26.8[ab]	24.0	7.68
6	7.36[a]	53.3[ab]	51.7[ab]	27.5[a]	22.7	7.25
7	7.33[ab]	50.7[b]	62.1[a]	24.2[b]	25.0	8.05
SEM[2]	0.0096	1.8320	2.7416	0.4800	0.9538	0.3184
P-value	0.0310	0.0300	0.0154	0.0141	0.6198	0.6002

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Treatments:1 = Broilers at 13.7 meters (45 ft) downwind from treatment 7; 2 = Broilers at 6.1 meters (20 ft) downwind from treatment 7; 3 = Broilers at 1.5 meters (5 ft) downwind from treatment 7; 4 = Broilers at 1.5 meters (5 ft) upwind from treatment 7; 5 = Broilers directly opposite treatment 7 and separated by a partition (41.9 cm high by 16.5 cm wide); 6 = Broilers penned with 4 MG inoculated layers; 7 = 4 MG inoculated layers. All pens size = 1.5 × 2.7 m (5 × 9 ft). [2]Pooled SEM for main effects (n = 16).

Table 2: Influence of MG inoculation on blood Ca^{2+}, K^+, Na^+ and Cl^- in inoculated hens and broiler chickens

Treatment[1]	Ca^{2+} (mEq/L)	K^+ (mEq/L)	Na^+ (mEq/L)	Cl^- (mEq/L)
1	3.05	4.81[b]	151[ab]	109
2	3.10	4.37[b]	151[ab]	109
3	3.07	4.96[b]	154[a]	111
4	4.94	4.90[b]	153[a]	110
5	3.14	5.11[b]	152[a]	109
6	3.05	4.73[b]	151[ab]	108
7	3.34	6.08[a]	148[b]	109
SEM[2]	0.7074	0.1427	0.7623	0.7302
P-value	0.4950	0.0014	0.0151	0.2295

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Treatments:1 = Broilers at 13.7 meters (45 ft) downwind from treatment 7; 2 = Broilers at 6.1 meters (20 ft) downwind from treatment 7; 3 = Broilers at 1.5 meters (5 ft) downwind from treatment 7; 4 = Broilers at 1.5 meters (5 ft) upwind from treatment 7; 5 = Broilers directly opposite treatment 7 and separated by a partition (41.9 cm high by 16.5 cm wide); 6 = Broilers penned with 4 MG inoculated layers; 7 = 4 MG inoculated layers. All pens size = 1.5 × 2.7 m (5 × 9 ft). [2]Pooled SEM for main effects (n = 16).

Table 3: Influence of MG inoculation on blood GLU, TRIG, Osmo and CHOL in inoculated hens and broiler chickens[1]

Treatment[1]	GLU (mg/dL)	Osmo (mmol/kg)	CHOL (mg/dL)	TRIG (mg/dL)	ANGAP (mmol/L)
1	240	316[ab]	119	168	20.5
2	233	315[ab]	119	127	19.9
3	250	321[a]	135	194	20.8
4	229	319[a]	125	155	19.9
5	230	318[ab]	120	147	21.3
6	232	314[ab]	133	115	20.0
7	228	309[b]	125	104	20.6
SEM[3]	8.5720	1.7515	5.0168	19.751	0.7295
P-value	0.5726	0.0230	0.2443	0.1235	0.7861

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Treatments:1 = Broilers at 13.7 meters (45 ft) downwind from treatment 7; 2 = Broilers at 6.1 meters (20 ft) downwind from treatment 7; 3 = Broilers at 1.5 meters (5 ft) downwind from treatment 7; 4 = Broilers at 1.5 meters (5 ft) upwind from treatment 7; 5 = Broilers directly opposite treatment 7 and separated by a partition (41.9 cm high by 16.5 cm wide); 6 = Broilers penned with 4 MG inoculated layers; 7 = 4 MG inoculated layers. All pens size = 1.5 × 2.7 m (5 × 9 ft). [2]Pooled SEM for main effects (n = 16).

(Ward, 2006). Oxygen depletion weakens the immune system, which may enhance viral infections. According to the World Health Organization conference on HIV, inactivation of HIV in patients will produce a significant rise in pO_2.

From a practical viewpoint, a bird's susceptibility to an infectious challenge can be subdivided into two components: resistance and resilience. Resistance refers to the capacity of a variety of anatomical and physiological systems, including the immune system, to exclude pathogens. However, resilience refers to the capacity of the bird to maintain productivity (e.g., growth, feed efficiency, egg production) during an infectious challenge.

An immune response requires extensive communication between a wide variety of body systems. The circulatory system plays important roles by modulating the release of communication molecules or by regulating the body homoeostasis through acid-base balance mechanism. Hence, the immune system does not function independent from other physiological systems, but is highly integrated with normal metabolism and physiology through the circulatory system. Communicable diseases are caused by *pathogens,* which can spread from one person or animal to another, either directly or through some other means. The immune system which includes blood plasma, lymph and lymphocytes, analyzes the chemical nature of the antigen and secretes a suitable "antibody" which may be natural or artificial (active acquired immunity) to detoxify the antigen.

Birds, like humans, rely on their immune systems to prevent severe clinical disease. Immune function is specifically linked to the release of O_2 radicals and the functioning of the immune system is stimulated by an increase of venous pO_2. When a disease agent, bacteria or virus, is presented to the immune system, it causes the immune system to react by activating the white blood cells and producing antibodies. These results are consistent with the hypothesis that the immunization/ vaccination process which is associated with acquired immunity is induced by pO_2. The ability of the immune

system to prevent disease can be enhanced by vaccination. In the vaccination process, the vaccine presents the antigen to the animal in a fashion that stimulates immunity without causing disease. From the physiological perspective, it could be concluded that in addition to protecting birds from MG infection, FMG vaccine may improve the layer chicken's ability to withstand the harmful effects of stressors on their performance and well-being due to an increase in pO_2. Indeed, poultry producers, in particular table egg producers, have stated that "F-strain MG vaccinated layers outperform mycoplasma-clean stock" (J. Self, V.P. of Operations, Cal-Maine Foods, Inc., Personal Communication). Further studies are needed to evaluate the effects of the observed changes in O_2 concentration on MG-infected birds.

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